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Cytotaxonomic Studies of *Thelypteris angustifrons* Complex (Thelypteridaceae)

NARUMI NAKATO[1], NORIO SAHASHI[2] and MASAHIRO KATO[3]

[1] Hijirigaoka High School, Tama University, 4-1-1 Hijirigaoka, Tama, Tokyo 206-0022, Japan; [2] Department of Biology, School of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan; [3] Department of Biological Sciences, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

We report for the first time a diploid cytotype ($2n = 54$) in the *Thelypteris angustifrons* complex from Okinawa Isl., the Ryukyus, although a hexaploid ($2n = 162$, $x = 27$) or a hyperhexaploid ($2n = 164$) has been reported from Japan (Kyushu and Honshu) and a diploid ($n = 31$) from Taiwan. The hexaploid is also found in Amami-oshima Isl. and more northern Japan. In addition, a triploid ($2n = 81$) and a hypotetraploid ($2n = 107$) are found in Amami-oshima Isl. and may be hybrids, because of irregular spore morphology. The spores of the diploids have less developed perispore and are smaller than those of the hexaploids. A variation in spore size of herbarium specimens examined shows that putative diploids occur only on Okinawa and Yakabi Isls. The diploid cytotype seems to be restricted to Okinawa and its neighboring area and also probably to Taiwan, while the hexaploid seems to be widely distributed from Taiwan north to warm temperate regions of Japan. From differences in chromosome number and spore and leaf morphologies, the diploid cytotype is regarded as *T. miyagii*, a species independent of the hexaploid *T. angustifrons*. The two related species, *T. glanduligera* and *T. cystopteroides*, were compared to the complex.

Key words: chromosome, distribution, polyploidy, spore, *Thelypteris angustifrons*, *Thelypteris miyagii*

Iwatsuki (1995) recognized 37 species in the genus *Thelypteris* sensu lato in the Japanese fern flora, of which 28 species have been reported for chromosome numbers (Takamiya 1996, Nakato 1998a, b). Among the cytologically investigated species, several species show variation in chromosome number and/or reproductive mode, which are regarded as species complexes. They include the *T. cystopteroides* (D. C. Eaton) Ching complex with sexual diploid and tetraploid ($x = 27$) (Kurita 1976, Kurizono 1987, Nakato 1998b), the *T. decursive-pinnata* (H. C. Hall) Ching complex with sexual diploid, sterile triploid and sexual tetraploid ($x = 30$) (Mitui 1966, Masuyama 1979), the *T. laxa* (Franch. & Sav.) Ching complex including sexual tetraploid ($2n = 136$, $x = 34$) and sexual hypotetraploid ($2n = 134$) (Nakato 1998a), the *T. phegopteris* (L.) Sloss. complex comprising sexual diploid and apogamous triploid ($x = 30$) (Matsumoto & Yano 1989, Hirabayashi 1989), and the *T. torresiana* (Gaudich.) Alston complex with sexual diploid and tetraploid ($x = 31$) (Nakato, in press) and so on. Cytological, morphological, and phytogeographical studies of such species aggregates provide useful infomation to understand their variation and speciation. We report here a polyploid series in *T. angustifrons* (Miq.) Ching, which is then considered to be a species complex like the above mentioned examples, and conclude that the complex includes two distinct sexual species.

Thelypteris angustifrons (Miq.) Ching [= *Parathelypteris angustifrons* (Miq.) Ching, *T. glanduligera* (Kunze) Ching var. *hyalostegia* sensu H. Ito (non Copel.), *T. glanduligera* var. *elatior* (D. C. Eaton) Sa. Kurata] is a small fern up to about 30 cm long, generally occurring in partially open places, e.g. at edges of forests, roadsides, and also on forest floors, and is distributed from China (Fujian and Zhejiang) to Taiwan and the Ryukyu Islands, north to Korea and southern and central Honshu of Japan (Kurata & Nakaike 1983, Lin *et al.* 1991, Zhang 1993, Iwatsuki 1995). Iwatsuki (1965) noted that *T. angustifrons* was extremely variable in the Ryukyu Islands, where there is a series of various forms including *Dryopteris miyagii* H. Ito and *D. okinawensis* H. Ito (Ito 1935), though these two species were reduced by Ito (1938) to *T. glanduligera* var. *hyalostegia* sensu H. Ito (= *T. angustifrons*). Serizawa (1975) also argued that plants with deeply dissected pinnae and sometimes lobed pinnules from Okinawa Isl., the Ryukyu Isls., might be separated as a distinct variety of *T. angustifrons*, and Ito's two species could be used for such a form. Nakaike (1975, 1992) treated *T. angustifrons* as a variety of *T. glanduligera* (var. *elatior*).

Although, as mentioned above, plants assigned to *Thelypteris angustifrons* from the Ryukyu Islands are greatly variable in external morphology, chromosomal investigations have not yet been conducted for them. We examined chromosome numbers and spores in plants of Okinawa Isl. and Amami-oshima Isl., the Ryukyus, as well as other northern regions of Japan. Additionally, *T. glanduligera* and the *T. cystopteroides* complex, both allied to the *T. angustifrons* complex, were also examined for chromosome numbers and spore and leaf morphologies to compare with those of the *T. angustifrons* complex.

Materials and Methods

Plant materials for chromosome observations were collected from the Ryukyus and the main islands of Japan (Table 1). Root tips were pretreated in 2 mM 8-hydroxyquinoline solution for 3-4 hr. They were fixed in 45% acetic acid for 10 min, macerated in 1N HCl at 60°C for 1 min, and stained and squashed in aceto-orcein solution. In order to infer reproductive mode, spore numbers per sporangium were counted in some specimens. In sexually-reproducing plants of the higher leptosporangiate ferns including *Thelypteris*, 64 spores are contained in each sporangium (Walker 1979). Spores from voucher specimens, which are deposited in the Herbarium, the Botanical Gardens, Graduate School of Science, University of Tokyo (TI), and herbarium specimens deposited in TI and KYO (the Herbarium, Department of Botany, Kyoto University) were embedded in Eukitt (O. Kindler, Germany), and the length, except for perispore, was measured for 100 samples per specimen using an ocular micrometer by light microscopy (LM). For scanning electron microscopic observations (SEM), spores were usually treated by the critical point drying method, spattered with gold and observed using an Akashi MSN-4 SEM at 10 kV.

Results and Discussion

Chromosome numbers

The chromosome counts obtained are summa-

TABLE 1. Localities, vouchers, chromosome numbers and mean spore lengths in *Thelypteris angustifrons* complex and its allied species examined.

Species / Locality	Voucher[1]	Chromosome number 2*n* (ploidy: *x* = 27)	Mean spore length ± SD (μm; *N* = 100)
***Thelypteris angustifrons* complex**			
Taniu-dake, Nago, Okinawa Isl., Okinawa Pref.	*2404-1*	54 (2*x*)	34.8 ± 2.6
Ogimi-son, Okinawa Isl., Okinawa Pref.	*2419-1*	54 (2*x*)	-
Yonahadake, Kunigami-son, Okinawa Isl., Okinawa Pref.	*2363*[2]	54 (2*x*)	36.9 ± 3.5
ibid.	*2415-1*[2]	54 (2*x*)	-
ibid.	*2418-1*[2]	54 (2*x*)	38.4 ± 2.1
ibid.	*2418-2*	54 (2*x*)	38.7 ± 2.3
Benoki, Kunigami-son, Okinawa Isl., Okinawa Pref.	*2407-1*	54 (2*x*)	36.3 ± 3.1
Naze, Amami-oshima Isl., Kagoshima Pref.	*2349*	81 (3*x*)	irregular
ibid.	*2318*	107 (hypo-4*x*)	irregular
ibid.	*2320*	162 (6*x*)	44.1 ± 2.9
Yamato-son, Amami-oshima Isl., Kagoshima Pref.	*2332*	162 (6*x*)	44.4 ± 3.4
Sumiyo-son, Amami-oshima Isl., Kagoshima Pref.	*2328*	162 (6*x*)	42.6 ± 3.7
Onoaida, Yakushima Isl., Kagoshima Pref.	*2293*	162 (6*x*)	41.1 ± 3.5
Yoshida-cho, Kagoshima-gun, Kagoshima Pref.	*2140*[2, 3]	162 (6*x*)	46.2 ± 2.9
Kyodomari, Ashikita-cho, Ashikita-gun, Kumamoto Pref.	*2375*[2]	162 (6*x*)	42.9 ± 3.3
Taguchi, Sanko-mura, Shimoge-gun, Oita Pref.	*2366*[2]	162 (6*x*)	43.8 ± 3.4
Usa-jingu, Usa, Oita Pref.	*2365*	162 (6*x*)	43.2 ± 3.4
Usukisekibutu, Usuki, Oita Pref.	*2424*	162 (6*x*)	43.8 ± 2.9
Mochidagaura, Hakata-ku, Fukuoka, Fukuoka Pref.	*2114*[2, 3]	162 (6*x*)	44.4 ± 2.7
Hirao-reien, Minami-ku, Fukuoka, Fukuoka Pref.	*2128*[3]	162 (6*x*)	45.0 ± 2.7
ibid.	*2129*[2, 3]	164 (hyper-6*x*)	43.2 ± 2.9
Hayashida, Tosayamada-cho, Kami-gun, Kochi Pref.	*2291*	162 (6*x*)	40.2 ± 2.8
ibid.	*2292*	162 (6*x*)	-
Takada, Shingu, Wakayama Pref.	*2102*[2, 3]	162 (6*x*)	46.2 ± 3.3
Iwane, Hosoe-cho, Inasa-gun, Shizuoka Pref.	*2307*	162 (6*x*)	-
Tuzukishinden, Mikkabi-cho, Inasa-gun, Shizuoka Pref.	*2314*	162 (6*x*)	44.4 ± 2.9
T. glanduligera			
Yamato-son, Amami-oshima Isl., Kagoshima Pref.	*2329*	108 (4*x*)	38.7 ± 2.5
Naze, Amami-oshima Isl., Kagoshima Pref.	*2352*	108 (4*x*)	-
Usukisekibutu, Usuki, Oita Pref.	*2369*	c.108 (4*x*)	39.0 ± 2.9
Tosayamada-cho, Kami-gun, Kochi Pref.	*2393*	108 (4*x*)	-
Takada, Shingu, Wakayama Pref.	*2103*[2, 3]	108 (4*x*)	39.6 ± 2.8
Tamako, Higashiyamato, Tokyo Pref.	*2091*	108 (4*x*)	38.7 ± 3.0
***T. cystopteroides* complex**			
Yuwandake, Amami-oshima Isl., Kagoshima Pref.	*2346*[2]	54 (2*x*)	39.0 ± 3.1
Naze, Amami-oshima Isl., Kagoshima Pref.	*2322*[2]	54 (2*x*)	37.5 ± 3.0
Misen, Miyajima Isl., Hiroshima Pref.	*2364*[2]	108 (4*x*)	43.2 ± 3.7

1) Nakato's specimen no.

2) Specimen with 64 spores per sporangium; the others are not examined.

3) The chromosome number was reported by Nakato (1998)

rized in Table 1.

Thelypteris angustifrons complex: Nakato (1998b) reported that eight plants from seven localities in Kyushu and Honshu are hexaploid with $2n = 162$ based on $x = 27$, and one plant from Kyushu has $2n = 164$ (hyperhexaploid). Tsai & Shieh (1977) recorded a different count of $n = 31$, diploid based on $x = 31$, from Nantou, Taiwan.

We examined 21 plants, of which seven plants were diploid with $2n = 54$, one was triploid with $2n = 81$, one was hypotetraploid with $2n = 107$ and the remaining 12 were hexaploid with $2n = 162$ (Table 1, Figs. 1A-D). These chromosome numbers except for the hexaploid are reported here for the first time in this complex. The three cytotypes occur in the Ryukyu Islands; the diploid was collected from Okinawa Isl. (Okinawa Prefecture), and the triploid and the hypotetraploid from Amami-oshima Isl. (Kagoshima Prefecture). In contrast, the hexaploid cytotype was found in the material from Amami-oshima Isl. and more northern localities. Figure 2 shows the distribution range of this complex and the localities of plants cytologically examined in the previous (Tsai & Shieh 1977, Nakato 1998b) and present studies. It is noted that three types, triploid, hypotetraploid and hexaploid, occur together on Amami-oshima Isl., situated between Okinawa Isl. and Yakushima Isl. where diploids and hexaploids occur, respectively. The triploid and hypotetraploid are very rare, the former produces abnormal shrunken spores, and the latter yields both irregular- and normal-shaped spores. Therefore they are presumed to be hybrids.

The count of diploid ($n = 31$) reported from Taiwan might be erroneous, because some chromosomes appear to be piled up in the photograph presented (Tsai & Shieh 1977, p. 93, Fig. 7). However, the Taiwanese plant is certainly considered as diploid from the photograph. Further chromosomal survey is needed for Taiwanese populations to confirm whether diploids based on $x = 27$ occur or not.

Thelypteris glanduligera: This species was reported to be tetraploid with $2n = 108$ ($x = 27$) in 14 plants from 13 localities in Japan (Nakato 1998b), whereas different chromosome numbers such as $n = 34$ and $n = 68$ ($x = 34$), $n = 36$ ($x = 36$) and $2n = 80$ ($x = 40$) were recorded from outside Japan (see Nakato 1998b). Five specimens from Kagoshima (Amami-oshima Isl.), Oita, Kochi and Tokyo Prefectures examined here showed $2n = 108$ (one counted $2n = c.$ 108), consistent with Nakato (1998b) (Fig. 1E).

Thelypteris cystopteroides complex: Two cytotypes, diploid ($2n = 54$) and tetraploid ($2n = 108$) based on $x = 27$, were recorded from Japanese populations (Kurita 1976, Kurizono 1987, Nakato 1998b). In the present study, two specimens from Amami-oshima Isl. were diploid with $2n = 54$, and one from Hiroshima Prefecture was tetraploid with $2n = 108$ (Figs. 1F, G).

Reproductive mode

Spore numbers per sporangium were counted in some voucher specimens (Table 1). All specimens examined of the three taxa were 64-spored, indicating that they reproduce sexually. The present result agrees with Momose (1967), who described prothallia (gametophytes) with normal antheridia and archegonia in the three species.

Spores of cytologically determined specimens

The results of measurements of spore lengths are shown in Table 1.

Thelypteris angustifrons complex: The Okinawa's diploid plants have monolete bilateral spores with almost smooth walls by LM. The mean spore lengths range between 34.8 and 38.7 μm in five specimens. From SEM observations, the outer perispores are smooth or occa-

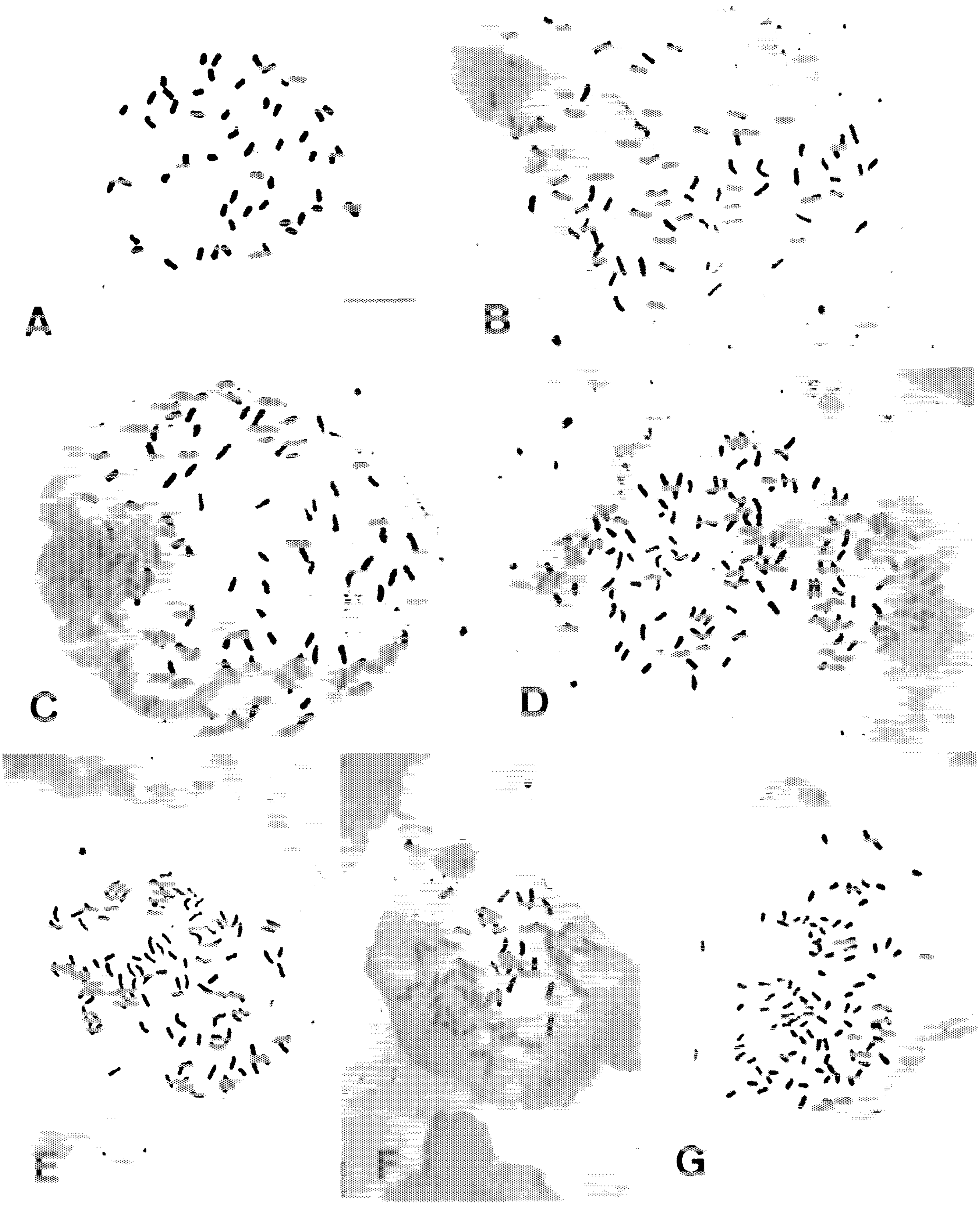

FIG. 1. Somatic chromosomes of *Thelypteris angustifrons* complex and related species. A-D. *T. angustifrons* complex. A. $2n = 54$, diploid (*Nakato 2234*). B. $2n = 81$, triploid (*Nakato 2349*). C. $2n = 107$, hypotetraploid (*Nakato 2318*). D. $2n = 162$, hexaploid (*Nakato 2332*). E. *T. glanduligera*, $2n = 108$, tetraploid (*Nakato 2329*). F-G. *T. cystopteroides* complex. F. $2n = 54$, diploid (*Nakato 2322*). G. $2n = 108$, tetraploid (*Nakato 2364*). Scale bar = 10 μm for all figures.

sionally ornamented by sparse linear muri (Figs. 3A, B). The triploid (*Nakato 2349*) and hypotetraploid (*Nakato 2318*) from Amami-oshima Isl. have irregular-shaped spores, presumably owing to abnormal meiosis; the triploid's spores are decorated with mostly immature and fragile perispore (Fig. 3F), but the hypotetraploid's spores are often normal-shaped, with the ornamentation of outer fragile perispore reticulate (Fig. 3G). The 15 hexaploids examined have larger spores (40.2-46.2 μm in mean length) with thin membranous perispore by LM. The inner perispore is almost smooth by SEM, while the outer perispore has linear muri, which partially form winglike reticulate ornamentations (Fig. 3C). The grand mean and standard deviation (SD) of spore length are 36.9 ± 3.1 μm in the diploid cytotype (five specimens, $N = 500$) and 43.8 ± 3.5 μm in the tetraploid (14 specimens, $N = 1400$). These two values differ sig-

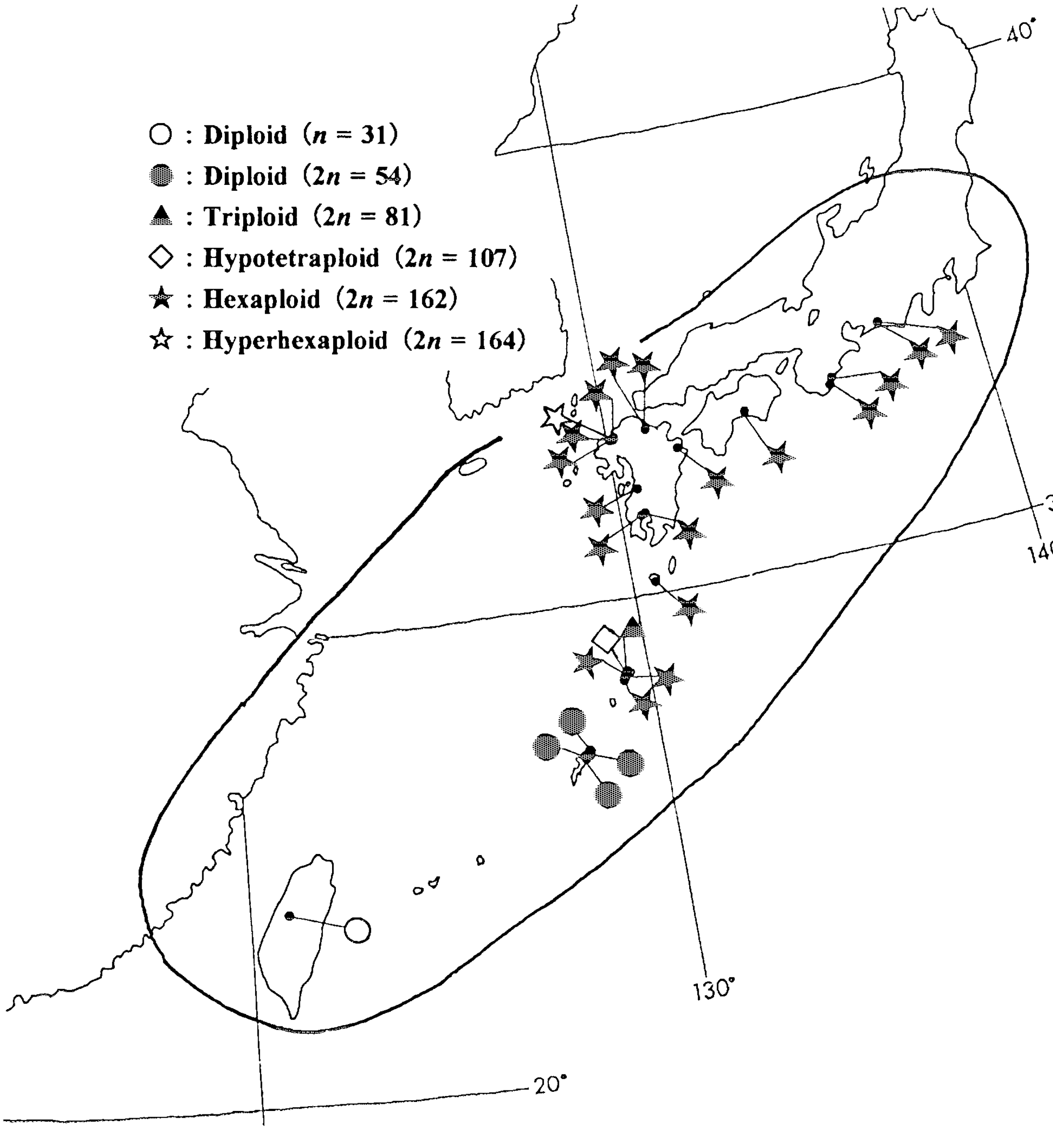

FIG. 2. Map showing distributions of cytotypes of *Thelypteris angustifrons* complex. Solid line indicates the distribution range of the complex. Data from Tsai & Shieh (1977), Nakato (1998) and the present study.

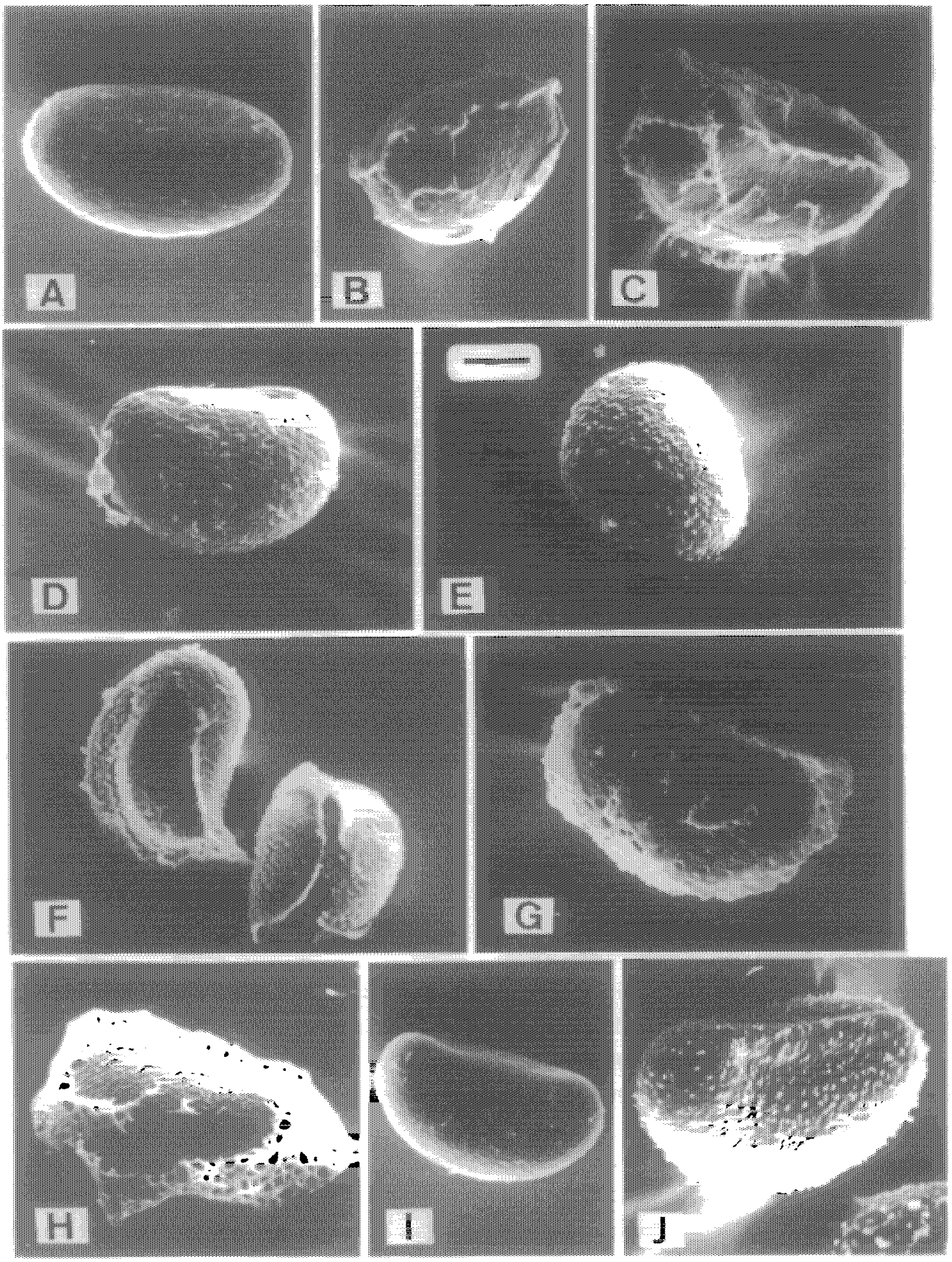

FIG. 3. SEM micrographs of spores of *Thelypteris angustifrons* complex and related species. A-G. *T. angustifrons* complex. A. Diploid (*Nakato 2415-1*). B. Diploid (*Nakato 2234*). C. Hexaploid (*Nakato 2102*). D. Type of *Dryopteris miyagii* (*Miyagi s.n.*). E. Type of *D. okinawensis* (*Miyagi s.n.*). F. Triploid (*Nakato 2349*). G. Hypotetraploid (*Nakato 2318*). H. *T. glanduligera*, tetraploid (*Nakato 2329*). I-J. *T. cystopteroides* complex. I. Diploid (*Nakato 2322*). J. Tetraploid (*Nakato 2364*). Scale bar = 10 μm for all figures.

nificantly by *t*-test ($p<0.001$).

Thelypteris glanduligera: In four specimens examined, the mean lengths of spores range between 38.7 and 39.6 μm ($N = 400$), and the grand mean and SD are 39.0 ± 2.8 μm, intermediate between the diploid and hexaploid of the *T. angustifrons* complex (Table 1). The perispores are membranous by LM. They are two-layered perispore by SEM; the inner layer shows plain surfaces, and the outer layer shows partly fimbriate wings with reticulate surface (Fig. 3H).

Thelypteris cystopteroides complex: The mean spore lengths of two diploid plants are 37.5 and 39.0 μm, with the grand mean and SD being 38.3 ± 3.5 μm, and those of one tetraploid are 43.2 ± 3.7 μm (Table 1). The tetraploid has larger spores than the diploids, with a significant difference between the two values (*t*-test, $p<0.001$). Although the spores of both cytotypes show smooth surfaces by LM, they are distinguished from each other in the ornamentation of perispore by SEM; the spores of the diploid individuals show plain surface ornamentation or nearly so with roughly minute granules, while those of the tetraploid have irregularly echinulate (Figs. 3I, J).

Spores of Thelypteris angustifrons *complex from herbarium specimens*

Because the above measurement of spores collected from cytologically determined specimens showed a significant difference in spore size between diploids and hexaploids, spores collected from herbarium specimens were further examined for their size in order to infer their ploidy level and know the distribution ranges of diploids and hexaploids. A total of 45 herbarium specimens were available for spore examination including the type specimens of *Dryopteris miyagii* H. Ito and *D. okinawensis* H. Ito, both reduced under *Thelypteris glanduligera* var. *hyalostegia* sensu H. Ito (= *T. angustifrons*) (Ito 1938). The results are shown in Figure 4 and Appendix. Specimens with irregular-shaped spores, suggesting hybrids, were not found. The mean spore lengths varied from 36.0 to 48.0 μm. The spores of the type specimens of *D. miyagii* and *D. okinawensis*, both from Okinawa Isl., are 36.3 and 36.0 μm, respectively, suggesting that they are most probably diploids. Furthermore, the spore wall morphology of the two types are similar to those of the diploid materials (Figs. 3D, E).

Putative diploid specimens with spores smaller than 39 μm in length occur in Okinawa Isl. (eight specimens) and Yakabi Isl. (one specimen), the Ryukyus, and putative hexaploids with spores larger than 41 μm occur widely from Okinawa Isl. and Kumejima Isl. in the Ryukyus north to central Honshu (Fig. 4). From a cytogeographical point of view, it is likely that the diploiod cytotype is restricted to Okinawa Isl. and its neighboring area as a relict. Polyploidization of this species complex certainly played an important role for northward expansion to warm temperate Japan.

For Taiwanese *Thelypteris angustifrons*, only a single specimen from Taipei was examined for spore size, and it was estimated to be hexaploid (mean spore length, 48.0 μm). Since Tsai & Shieh (1977) reported diploid chromosome number ($n = 31$) from Nantou, different cytotypes perhaps exist in Taiwan.

Leaf morphology of Thelypteris angustifrons *complex*

The individuals assigned to the *Thelypteris angustifrons* complex are distinguishable from the closely allied *T. glanduligera* and *T. cystopteroides* complex by leaf morphology. They differ from *T. glanduligera* (Fig. 5F) in having smaller leaves, oblong to oblong-subdeltoid pinnae, 1-2 shortened pairs of basal pinnae, 1-3 free acroscopic basal pinnules, and reniform or rather irregular-formed indusia (Iwatsuki 1965, 1995, Kurata & Nakaike 1983). They also differ from the *T. cystopteroides* complex (Figs. 5G, H) in the rhizomes not so slender, laminar surface pubescent, and pinnules or segments more or less acute at the apex (Iwatsuki 1965, 1995, Kurata & Nakaike 1983).

Plants of the *Thelypteris angustifrons* complex in the Ryukyus are variable in leaf characters, as pointed out by Ito (1935, 1938), Iwatsuki (1965) and Serizawa (1975). Figures 5A and B show fertile pinnae of two diploid specimens. The specimen *Nakato 2415-1* (Fig. 5A) has deeply dissected pinnae with 2-3 pairs of basal pinnules free, incised, while *Nakato 2407-1* (Fig. 5B) has pinnae with one pair of basal free, almost entire pinnules. The former is very similar to the type of *Dryopteris miyagii*, and the latter to that of *D. okinawensis* (Figs. 6A, B).

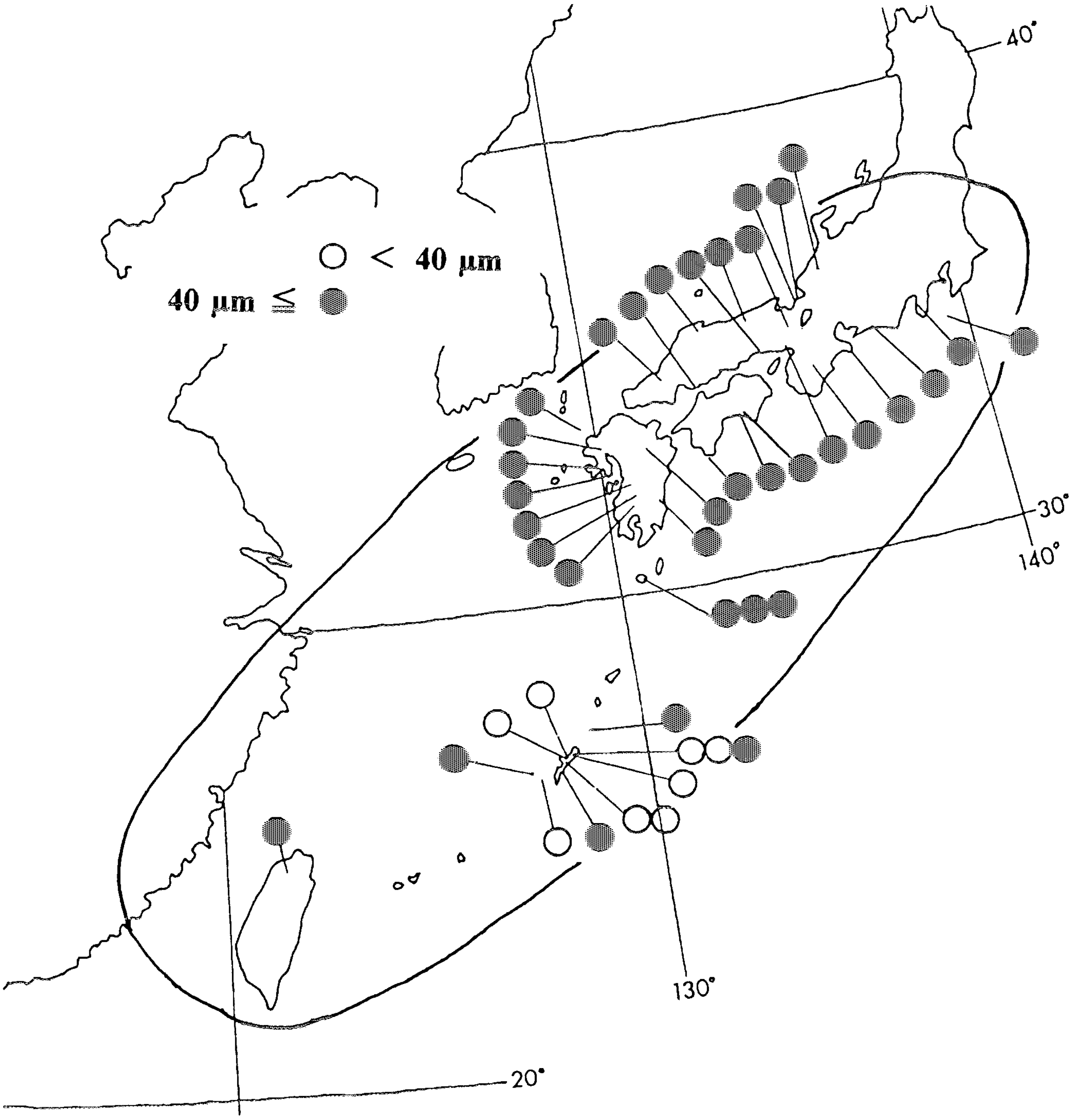

FIG. 4. Map showing distributions of *Thelypteris angustifrons* complex with two classes of spore size. Symbols indicate mean spore lengths, and solid line indicates the distribution range of the complex.

Furthermore, there are successively intermediate leaves quite often in the other diploid vouchers. In comparison, the hexaploid plants examined in the present study have single elliptic to ovate free basal pinnule (sometimes shallowly lobed) on the acroscopic side in middle and lower pinnae (Fig. 5E), though the leaf sizes are variable according to plants. Therefore, the diploid is usually distinguishable from the hexaploid in having more deeply dissected pinnae and free 1-3 acroscopic basal pinnulae with incised margins. Although diploids with leaf morphology of the *D. okinawensis* type do not seem to occur abundantly as compared with *D. miyagii* and the intermediate types, they are so similar to the hexaploid in the shape of segments, that it is

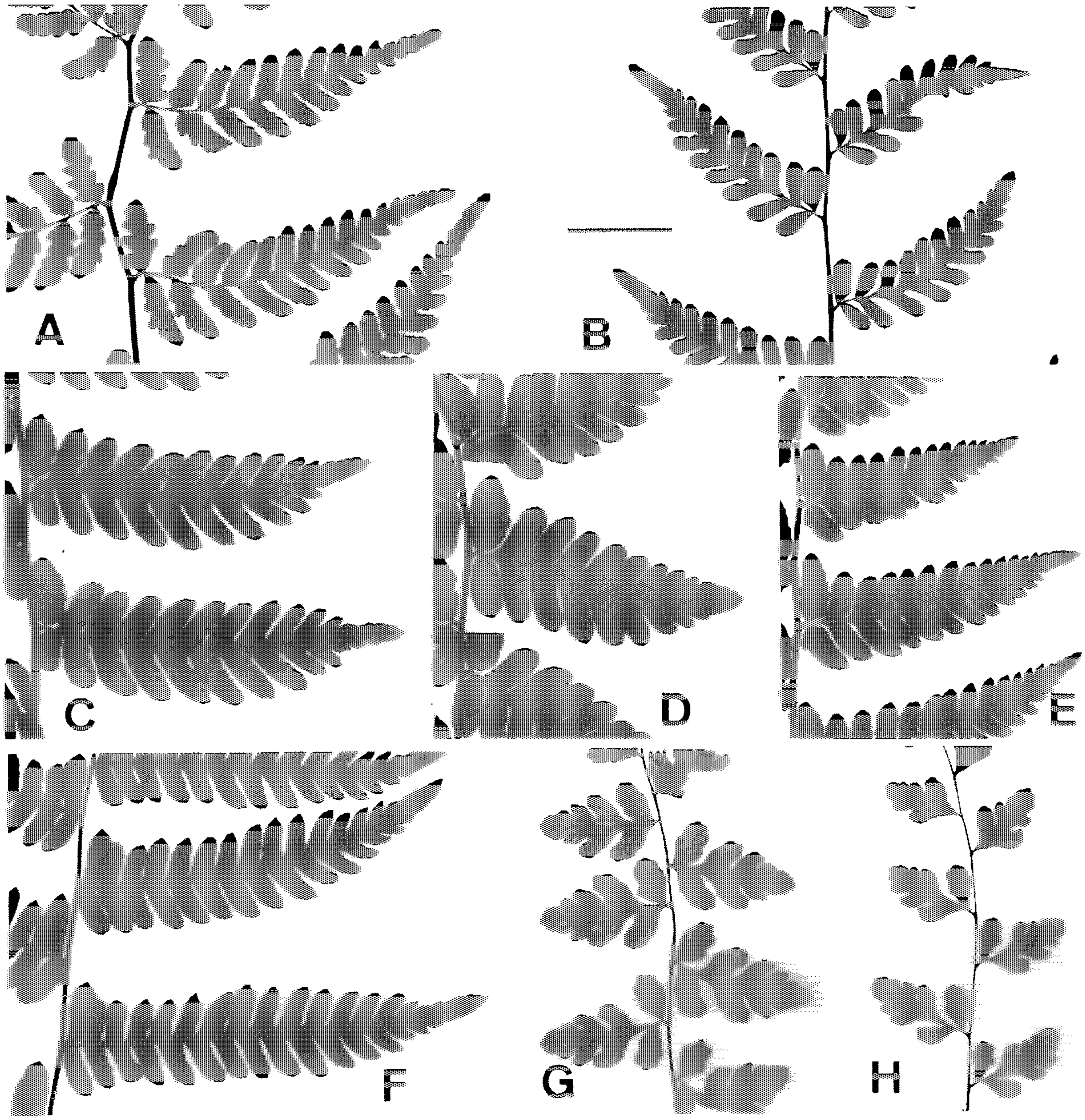

FIG. 5. Fertile middle pinnae of *Thelypteris angustifrons* complex and related species. A-E. *T. angustifrons* complex. A. Diploid (*Nakato 2415-1*). B. Diploid (*Nakato 2407-1*). C. Triploid (*Nakato 2349*). D. Hypotetraploid (*Nakato 2318*). E. Hexaploid (*Nakato 2293*). F. *T. glanduligera*, tetraploid (*Nakato 2393*). G-H. *T. cystopteroides* complex. G. Diploid (*Nakato 2322*). H. Tetraploid (*Nakato 2364*). Scale bar = 1 cm for all figures.

difficult to distinguish the two only by leaf morphology. In external morphology, the triploid, hypotetraploid and hexaploid are so similar to each other that it is difficult to distinguish them only by leaf characters.

As to the herbarium specimens examined for spore size, the putative diploids from Okinawa and Yakabi Isls. show as great variation in leaf morphology as the diploid vouchers. For example, specimens from Mt. Taniu-dake (*Nishida R-166, R-199*) have incised segments as *Dryopteris miyagii*, and that of Genka-gawa (*Nozu s.n.*) has non-incised pinnulae as *D. okinawensis*. The other putative diploids (four specimens) show intermediate forms with free 1-2 basal acroscopic pinnules shallowly lobed. On the other hand, most of the putative hexaploids from the Ryukyus (Kume-jima Isl., *Nozu s.n.*; Okinawa Isl., *Tagawa 46*; Yoron Isl., *Tagawa & Iwatsuki 2520*) and Taiwan (*Shimada 6111B*) have single free, almost entire basal pinnules as in the hexaploid vouchers, but one from Okinawa Isl. (Benoki-yama, *Ito s.n.*) shows intermediate morphology between *D. miyagii* and *D. okinawensis*. The other putative hexaploids from Yakushima Isl. and more northern areas of Japan have similar leaf morphology to the hexaploid vouchers.

Taxonomic treatment

As discussed above, the Okinawa's individuals of the *Thelypteris angustifrons* complex differ from the plants in Amami-oshima Isl. and more northern Japan in ploidy level, the size and morphology of spores, and leaf morphology. Therefore, the diploid cytotype is recognized as a species, different from the hexaploids of the complex. The diploid cytotype is certainly assignable to *Dryopteris miyagii* and *D. okinawensis* described from Okinawa. From observations of a variation of leaf morphology, we conclude that the two represent extreme forms of a single species with variable leaf morphology. Because the description of *D. miyagii* appeared earlier than that of *D. okinawensis* in Ito's paper (1935), the name of *D. miyagii* has a priority for publication. *Thelypteris angustifrons*, which was originally described from Nagasaki Prefecture, Kyushu, and is located within the range of the species, should be named for the hexaploid. The type of *T. glanduligera* var. *elatior* (D. C. Eaton) Sa. Kurata (Nakaike 1992), a synonym of *T. angustifrons*, was collected from Amami Islands.

In conclusion, the *Thelypteris angustifrons* complex is classified into two species by the features summarized in the following key:

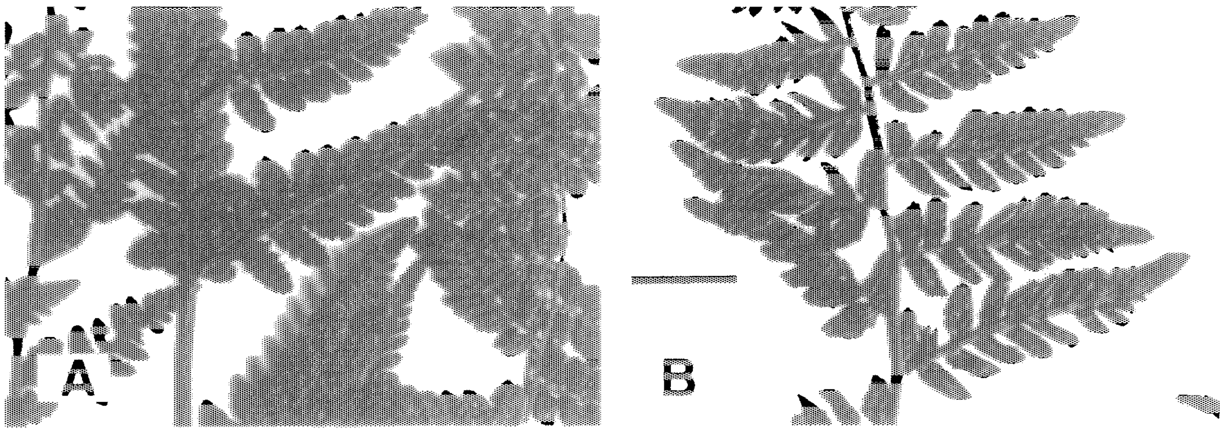

FIG. 6. Types of *Dryopteris miyagii* (A; *Miyagi s.n.*, TI) and *D. okinawensis* (B; *Miyagi s.n.*, TI). Scale bar = 1 cm.

1. Pinnules incised or entire, 1-3 basal pairs free; spores smooth or ornamented with sparse linear muri, < 40 μm long (mean); diploid ($2n = 54$).. *T. miyagii*

1. Pinnules entire or shallowly lobed, basal acroscopic pinnule free; spores ornented with linear muri partially reticulate, ≧ 40 μm long (mean); hexaploid ($2n = 162$) ....*T. angustifrons*

Thelypteris miyagii (H. Ito) Nakato, Sahashi & M. Kato, **comb. nov.**

Dryopteris miyagii H. Ito, Bot. Mag. Tokyo 49: 361 (1935). —*Type*: *Miyagi s.n.* (TI), Okinawa Isl.: Genka, Aug. 16, 1911.

Dryopteris okinawensis H. Ito, Bot. Mag. Tokyo 49: 361 (1935), *syn. nov.*—*Type*: *Miyagi s.n.* (TI), Okinawa Isl.

Thelypteris glanduligera var. *hyalostegia* sensu H. Ito, Bot. Mag. Tokyo 52: 589 (1938), p.p.; non Copel.

Thelypteris glanduligera (Kunze) T. Moore var. *elatior* (D. C. Eaton) Sa. Kurata, in Namegata & Kurata, Enum. Jap. Pterid. 343 (1961), p.p.; Nakaike, Enum. Pterid. Jap. 297 (1975), p.p. —*Parathelypteris glanduligera* (Kunze) Ching var. *elatior* (D. C. Eaton) Nakaike, Fl. Jap. rev. & enlarg. ed., 561, 817 (1992), p.p.

Thelypteris angustifrons (Miq.) Ching, Bull. Fan Mem. Inst. Biol. 6: 318 (1936), p.p.; Iwatsuki, Mem. Coll. Sci. Univ. Kyoto, B, 31: 168 (1965), p.p.; Serizawa, Sci. Rep. Takao Mus. Nat. Hist. 7: 46 (1975), p.p. — *Lastrea miqueliana* Tagawa, Acta Phytotax. Geobot. 15: 14 (1953), p.p. —*Parathelypteris angustifrons* (Miq.) Ching, Acta Phytotax. Sin. 8: 302 (1963), p.p.; Hatusima, Fl. Ryukyu, 179 (1975), p.p.; Walker, Fl. Okinawa & S. Ryukyu Isls., 91 (1976), p.p; Tsai & Shieh, Fl. Taiwan. 2nd ed. 1: 386 (1994), p.p.; Shing *et al.*, Fl. Reip. Pop. Sin. 4(1): 41 (1999), p.p.

Japanese name: Ryukyu-hashigoshida (Ito 1935).

Distribution: Okinawa Isl., probably also neighboring islands and Taiwan.

Habitat: On open forest floor or sunny slopes along road.

Chromosome number: $2n = 54$ (diploid).

The origin of the hexaploid *Thelypteris angustifrons* is uncertain and needs further study. It is possible that the hexaploid evolved via polyploidization from a triploid hybrid, which appeared between *T. miyagii* and a tetraploid species (e.g. *T. glanduligera*). *Thelypteris angustifrons* and *T. glanduligera* are sometimes hardly distinguishable (Iwatsuki 1965, 1995) and Nakaike (1975, 1992) treated them as conspecific varieties.

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Appendix

Herbarium specimens in TI and KYO, and their spore sizes (in parentheses mean spore length ± SD, μm; *N* = 100).

Thelypteris angustifrons

Taiwan: Taipei, *Shimada 6111B* (KYO) (48.0 ± 3.7). **Ryukyu**: Okinawa Prefecture: Kume-jima Isl., *Nozu s.n.* (TI) (47.7 ± 4.1); Yakabi Isl., *Shimabuku 1685* (KYO) (36.9 ± 3.4); Higashi-onna, Nakagami-gun, Okinawa Isl., *Tawada 46* (KYO) (43.8 ± 3.5); Mt. Taniu-dake, Okinawa Isl., *Nishida R-166* (TI) (37.8 ± 1.9), *R-199* (TI) (36.9 ± 2.9); Genka-gawa, Hanechi-mura, Okinawa Isl., *Nozu s.n.* (TI) (38.4 ± 2.3); Mt. Yonaha-dake, Kunigami-gun, 400–490m alt., Okinawa Isl., *Koyama, Fukuoka & Kato 618* (TI) (37.2 ± 3.8); Benoki-ken-yurin, Kunigami-mura, Kunigami-gun, Okinawa Isl., *Tsuyama 135* (TI) (38.4 ± 3.8); Benoki-yama, Kunigami-gun, Okinawa Isl., *Ito s.n.* (TI) (42.9 ± 3.2); Between Benoki and Ada, Kunigami-gun, Okinawa Isl., *Tsuyama 204* (TI) (37.5 ± 2.6). Kagoshima Prefecture: Gusuku, Yoron Isl., *Tagawa & Iwatsuki 2520* (KYO) (45.6 ± 3.2); Yamato-son, Amami- oshima Isl., *Nakato 2330* (TI) (45.9 ± 3.4), *2334* (TI) (45.6 ± 3.4). **Kyushu**: Kagoshima Prefecture: Yakushima Isl., *Muramatsu s.n.* (TI) (47.1 ± 3.8); Yakushima Isl., *Masamune s.n.* (TI) (44.7 ± 4.6); Nagata - Nagatazaki, Yakushima Isl., *Iwatsuki 3216* (KYO) (46.2 ± 3.3); Fuke, Yamano-mura, *Muramatsu s.n.* (TI) (45.0 ± 3.8); Ara-gochi in Tashiro, west of Oguchi, *Tagawa & Iwatsuki 3563* (KYO) (42.9 ± 3.5). Miyazaki Prefecture: Shimokita, Miyazaki, *Miyazawa 77* (TI) (44.1 ± 3.2). Oita Prefecture: Toriya, Kusu-gun, *Ikebe 6030B.* (KYO) (45.9 ± 3.8). Nagasaki Prefecture: Nomo - Mt. Gongenyama, Nomozaki-cho, Nishisonogi-gun, *Fujita & Mitsuta 426* (KYO) (44.7 ± 3.7); Mt. Inasayama, Nagasaki-shi, *Maekawa 89-12* (TI) (46.5 ± 4.0); Mt. Kurokami-yama, *Yamashita 60* (TI)(42.0 ± 3.6); Iki Isl., *Kinashi 14* (TI)(43.5 ± 3.4). **Chugoku**: Yamaguchi Prefecture: Kurokamijima, Tonda, Tsuno-gun, *Migo s.n.* (KYO) (45.6 ± 3.3); Nanmyouji-yama, Tsubaki-mura, Abu-gun, *Nikai s.n.* (TI) (44.4 ± 3.1). Tottori Prefecture: Tottori, *Yoshida s.n.* (KYO) (44.7 ± 3.4). Shimane Prefecture: Wanibuchidera, Hirata-cho, *Nozu s.n.* (TI) (44.1 ± 2.9). **Shikoku**: Kochi Prefecture: Okino-shima, Hata-gun, *Tagawa 137* (KYO) (46.2 ± 4.0); Ioki, Aki, *Naruhashi & Wakabayashi 203* (KYO)

(42.9 ± 2.6); Nakano-kawa, Yasuda-cho, Aki-gun, *Tagawa 145* (KYO) (45.0 ± 3.3). **Kinki**: Hyogo Prefecture: Yamamoto in Settu, *Togashi TNS 1544* (TI) (43.2 ± 2.9); Syosya-zan, n.w. of Himeji, *Tagawa 4942* (KYO) (45.9 ± 3.2). Nara Prefecture: Mt. Kasuga, Nara, *Tagawa 4202* (KYO) (44.7 ± 3.7). Kyoto Prefecture: Eastern slope of Mt. Mitokesan, Funai-gun, *Mitsuta 1570* (KYO) (44.4 ± 2.8). Mie Prefecture: Arajima, Shima, *Magofuku 7* (TI) (41.7 ± 3.5). **Chubu**: Fukui Prefecture: Shiroyama, Takahama-cho, *Murata 27396* (KYO) (47.1 ± 3.6); Ohshima, Ohi-gun, *Hori s.n.* (KYO) (42.0 ± 3.0); Hinoyama, Imadate-gun, *Hori s.n.* (KYO) (44.7 ± 3.0). Shizuoka Prefecture: Toyoda-mura, Shizuoka, *Muramatsu s.n.* (TI) (44.1 ± 3.2); Oosezaki, Izu, Teramoto s.n. (TI)(46.8 ± 3.4). **Kanto**: Tokyo Prefecture: Kozu-shima Isl., *Ohba 2990* (TI) (46.2 ± 4.6).

Dryopteris miyagii

Ryukyu: Type specimen: Genka, Okinawa Isl., *Miyagi s.n.* (TI) (36.3 ± 2.3).

Dryopteris okinawensis

Ryukyu: Type specimen: Okinawa Isl., *Miyagi s.n.* (TI) (36.0 ± 3.2).

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